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FARM CROPS AND SOILS

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Many investigators have studied the organic matter in soils, in the attempt to determine its nature and composition, the rate and character of the decomposition which it undergoes, the products of decomposition, and especially the value of various kinds of organic materials which may be applied to soils, as indicated by decomposition studies.

When carbon dioxide was first discovered in the soil, the theory was advanced that it resulted from the decomposition of organic matter. This suggested a new line of study and the production of carbon dioxide in the soil became the subject of many investigations. Various methods have been employed and information of much value has been obtained but there are still many problems, the solution of which awaits the development of new methods of study.

Standard methods for the study of the various problems in this field are much needed. It is the purpose of the work reported here to review the methods which have been employed in previous studies and to make a critical study of some of them.

HISTORICAL

The methods which have been employed in the study of carbon dioxide production in soils may be conveniently grouped as follows: (a) those involving the determination of the concentration of carbon dioxide in the soil air, (b) those measuring the evolution of carbon dioxide from soils, and (c) those determining the diffusion of carbon dioxide through soils.

DETERMINATION OF THE CONCENTRATION OF CARBON DIOXIDE IN SOIL AIR

The carbon dioxide content of the soil air was first determined by Boussingault and Lewy (3). The sample of air for analysis was taken at a depth of 30 to 40 cm. by means of a tube at the end of which was placed a perforated bulb filled with small, round pieces of quartz. The tube was placed in the soil and left 24 hours before starting the aspiration. Two to ten liters of the soil air were drawn from this tube and bubbled slowly through barium hydroxide, the rate of aspiration

being about 1 liter per hour. This method or some modification of it has been employed by many investigators. Pettenkofer (17) employed essentially the same method except that larger samples of air were taken in a shorter time.

Schloesing (23), Russell and Appleyard (22), Potter and Snyder (20) and Appleman (1) employed narrow, perforated pipes placed in the ground from which small samples were taken by a mercury pump and analyzed in the laboratory. Lundegardh (13, 14), and Jodidi (8) took small samples of air from such pipes in the field and made analyses with a portable volumetric apparatus. The tubes or pipes used by these investigators were of different kinds, some being made of steel, some of brass and others of glass; some were placed permanently and others were portable.

Leather (10) took a core of soil by means of a special cylinder and extracted the gases for analysis with a vacuum pump, using a volumetric analysis apparatus for determining the carbon dioxide.

THE MEASUREMENT OF THE EVOLUTION OF CARBON DIOXIDE FROM SOILS

Peterson (18), Deherain and Demoussy (5) and Wollny (30) studied the carbon dioxide-producing power of soils in the laboratory. Small samples of soil were placed in tubes and incubated at constant temperature for various periods of time, carbon dioxide-free air being drawn through the tubes at specified rates or the tubes were exhausted with a vacuum pump and the carbon dioxide determined either volumetrically or gravimetrically.

Stoklasa and Ernest (27) measured the amount of carbon dioxide evolved from a unit weight of soil in a given time, expressing the result as milligrams of carbon dioxide per kilogram of soil per day which they referred to as the "Respiration Intensity" of the soil. The method employed involved the incubation in large glass cylinders, of 1 kilogram portions of soil, at a given temperature and moisture content, and the determination of the carbon dioxide evolved in 24 hours. The cylinders were aerated by continuous aspiration through the soil at the rate of 20 liters in 24 hours, the air being washed free of carbon dioxide before it entered the cylinders, and the carbon dioxide evolved from the soil being absorbed in potassium hydroxide and weighed.

The method with slight modifications has been employed by Sewerin (25), Van Suchtelen (28), Lemmerman (11), Neller (15), Potter and Snyder (20), Waksman and Starkey (29) and Marsh (15). Further adaptations of the method for studying

the evolution of carbon dioxide from soils *in situ* have been proposed by Stoklasa and Doerell (26) and Humfeld (6).

Leather (10) adapted the method of Deherain and Demoussy (5) for determining the amount of carbon dioxide produced by the soil. He placed 100 grams of soil in a bottle and measured volumetrically the accumulation of carbon dioxide after 22 days. Lundegardh (13, 14), modified the method further by using a 1 liter Erlenmeyer flask and an incubation period of 24 hours. This method was considered superior to those involving aeration and suction for obtaining the sample of air for analysis.

DETERMINATION OF THE RATE OF DIFFUSION OF CARBON DIOXIDE THROUGH SOIL

Buckingham (2) studied the diffusion velocity of carbon dioxide through the soil and derived a formula from which the rate of production could be calculated. Leather (10) applied Buckingham's formula and compared the calculated quantities of gases diffusing per day. The quantity of carbon dioxide formed per 100 grams of soil per day was calculated to be 0.42 cc., whereas by experiment, using 100 grams of soil in a bottle after 22 days, 1.4 cc. of carbon dioxide was produced per day. He concluded that while the conditions were perfectly defined in the calculation, it was quite impossible to define those occurring in the soil due to the rapid changes in temperature, moisture content of the soil and concentration of carbon dioxide in the soil air.

Lundegardh (13, 14), measured the carbon dioxide evolved from the surface of the soil *in situ*, which he called "Soil Respiration," and the concentration of carbon dioxide at different depths and calculated the diffusion value (K) of a stratum of the soil. Since the oxygen absorbed is approximately equal to the carbon dioxide evolved this diffusion value serves as a very good measure for the aeration of the soil.

EXPERIMENTAL

The concentration of carbon dioxide in the soil air at any given time is a resultant of many factors and alone cannot be regarded as directly proportional to the rate of production. It is, however, a function of the rate of production, the rate of escape, solution, utilization, adsorption or combination, and, therefore, measurements of the concentration of carbon dioxide in the soil air should be made with these considerations in mind. Such measurements may indicate fairly accurately the rate of production of carbon dioxide in the soil and may also show something regarding the aeration of soils.

PART I. PERCENTAGE OF CARBON DIOXIDE IN SOIL AIR

The methods which have been employed for the determination of the concentration of carbon dioxide in the soil air have consisted, in general, in taking a large sample for gravimetric or titrametric determination or a small sample for volumetric analysis. Large samples were taken by aspiration and the carbon dioxide absorbed either in sodium, potassium or barium hydroxide. Carbon dioxide was then determined either titrametrically or gravimetrically.

When the carbon dioxide was absorbed in sodium hydroxide, it was determined by double titration. When potassium hydroxide was used as the absorbent various methods were employed to determine the carbon dioxide. Among these were double titration, precipitation of the carbon dioxide as barium carbonate and titration of the excess potassium hydroxide, weighing the barium carbonate, or simply a determination of the increase in weight of the solution. In the volumetric determination of the concentration of carbon dioxide in the soil air either a small sample was taken to the laboratory for analysis or a portable apparatus was set up in the field. Comparative studies were made on field and greenhouse soils by the gravimetric, titrametric and volumetric methods, using a modified portable Haldane gas analysis apparatus for the volumetric determinations.

The recovery of carbon dioxide from a sample of sodium carbonate was studied by several gravimetric and titrametric methods to determine some of the factors affecting the accuracy of the results. Pure anhydrous sodium carbonate was used with sulfuric acid in a Knorr alkalimeter. A typical set of results secured by the different methods is presented in table I.

Complete absorption of the carbon dioxide was secured in potassium hydroxide in a Geissler bulb. The results with soda-lime were always low, indicating incomplete absorption. This

TABLE I. RECOVERY OF CARBON DIOXIDE FROM SODIUM CARBONATE.

Method of determination	Mgm. CO_2 in sample	
	Calculated	Recovered
Potassium hydroxide in Geissler bulb. Gravimetric	415.1	414.8
Soda-lime in Flemming bulb. Gravimetric	415.1	396.0
Ascarite in Fisher bulb. Gravimetric	415.1	415.0
Potassium hydroxide, carbonate precipitated with barium chloride and the excess potassium hydroxide titrated	415.2	421.2
Barium hydroxide in Truog Tower. Titration.	440.0	390.5

was probably due to the large amount of carbon dioxide present at one time. Ascarite proved to be an efficient absorbent. The titration of potassium hydroxide solution, either by the double titration or titrating the excess after precipitating the bicarbonate with barium chloride, gave unsatisfactory results. Barium hydroxide in Truog towers was unsatisfactory for recovering carbon dioxide liberated from sodium carbonate, probably because the carbon dioxide was evolved too rapidly for complete absorption.

The percentage of carbon dioxide in the soil air was determined by gravimetric and volumetric methods in soils in the laboratory and in the field. Preliminary work indicated that much of the difficulty in determining the percentage of carbon dioxide in the soil air consisted in securing a representative sample of the air for analysis. The results secured with a soil tube representative of one kind generally employed (13, 14), proved unsatisfactory. It was necessary to have a tube which would be used for both the gravimetric and volumetric determinations in order to secure satisfactory comparisons. For this purpose a soil tube of about 300 cc. capacity was found very satisfactory (fig. 1).

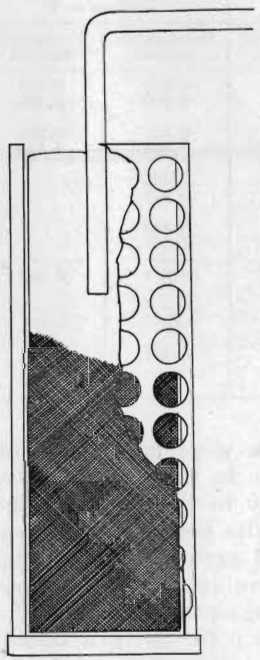

Fig. 1. Soil tube.

This tube consisted of a brass pipe 2 inches in diameter and 6 inches long with a large number of ½-inch holes drilled in it. It served as a skeleton for another tube made from fine mesh brass screen which was fitted inside it. One end of the outer tube was closed with a solid piece of brass, and a brass pipe ⅛-inch in diameter and 18 inches long led out of the other end. This tube was planted horizontally in the 6-8 inch layer of soil. A representative, small sample could be taken from this tube for volumetric analysis without the aid of much suction, and a fairly large sample by aspiration without dilution from the outside air. The tube was always planted 2 or 3 weeks before the experiment began.

Tama silt loam was sieved to pass a ¼-inch screen and placed in 4-gallon pots in the laboratory studies. Soil tubes were located in the 6-8 inch surface layer of soil. Two pots were treated with calcium hydroxide at the rate of 3 tons per acre and 2 pots were left untreated.

The moisture content was adjusted to approximately 25 percent and maintained at this content by frequent additions of distilled water.

The percentage of carbon dioxide in the soil air of the check and maintained at this content by frequent additions of disly and volumetrically. Fifty cc. of air were withdrawn with a hard rubber syringe and discarded, then three 10-cc. samples were taken in the Haldane apparatus for analysis. For gravimetric determinations the air was first passed through two U-tubes filled with calcium chloride which had been saturated with carbon dioxide, then into ascarite and a calcium chloride guard tube. A wet test gas meter was placed in the train to determine the volume of the sample and a flow meter to regulate the rate of aspiration. A vacuum pump was used for aspiration; the rate was 5 liters per hour. The results secured are presented in table II.

TABLE II. CARBON DIOXIDE IN THE SOIL AIR IN TAMA SILT LOAM IN POTS

Series	Volume of sample cc.	Percent CO_2 in air in untreated soil		Percent CO_2 in air in soil treated with 3 tons of lime per acre	
		Gravimetric method	Volumetric method	Gravimetric method	Volumetric method
1	500	0.259 0.235	0.196 0.170	0.200 0.259	0.195 0.195
2	1,400	0.297 0.242	0.300 0.253	0.331 0.403	0.355 0.420
3	1st 500 2nd 500	0.411 0.289		0.385 0.385	
	1st 1,000 2nd 1,000	0.211 0.163		0.349 0.367	
	Average	0.268	0.265	0.371	0.360
4	1st 5,600 2nd 5,600	0.249 0.209	0.225 ——	—— ——	—— ——
5	1st 14,100 2nd 14,100	0.198 0.170	0.220 ——	0.391 ——	0.310 ——

In series 1, in which 500-cc. samples were used, the data giving the percentage of carbon dioxide in the soil air determined gravimetrically varied irregularly in the different pots and were somewhat higher than the results secured by the volumetric method. There was a very good agreement in the percentage of carbon dioxide determined volumetrically. Closer agreement between the two methods of analysis was secured in series 2 when 1,400-cc. samples were taken for the gravimetric determinations, but the volumetric results were somewhat variable. Two successive 500-cc. samples and two successive

1,000-cc. samples were taken from one limed and one check soil for the gravimetric determinations in series 3. The percentage of carbon dioxide in the air of the check soil was high in the first 500-cc. sample and diminished in each successive sample. The average percentage of carbon dioxide for the 3-liter composite sample was 0.268 with the gravimetric method compared with 0.265 for the volumetric determination. In the limed soil there was much less variation between the results secured gravimetrically on the successive samples than were found in the check soil. The average percentage of carbon dioxide in the composite 3-liter sample was 0.371 compared with 0.360 by the volumetric method.

Two successive 5,600-cc. samples were taken from the check soil for the gravimetric analysis in series 4. The average percentage of carbon dioxide in the soil air was 0.229 by the gravimetric method and 0.225 determined volumetrically. Two successive samples of 14,100 cc. were taken from the check soil and only one sample of 14,100 cc. from the limed soil for the gravimetric determinations in series 5. The results showing the percentage of carbon dioxide in air of the check soil were slightly lower when the gravimetric method of determination was used than when the volumetric method was employed. The reverse was true, however, in the limed soil when only one sample of 14,100 cc. was taken.

These results as a whole indicate that a 3 to 6-liter sample of the soil air when carefully taken for gravimetric determinations may give results comparable with those secured by volumetric methods. The chances for error, however, are multiplied many times in the gravimetric determinations. Also the method is laborious and time consuming. The same soils were sampled on several different dates, 1,000-cc. samples being taken for the gravimetric analysis for comparison with the volumetric determinations. The results are presented in table III.

There is no agreement in the percentage of carbon dioxide in the soil air by the two methods. At one sampling the percentage

TABLE III. THE CARBON DIOXIDE IN THE SOIL AIR.

Date of sampling	Percent CO_2 in air in untreated soil		Percent CO_2 in air in soil treated with 3 tons lime per acre	
	Gravimetric method	Volumetric method	Gravimetric method	Volumetric method
2/19	———	0.180	0.267	0.190
2/25	0.174	0.262	0.186	0.287
2/26	———	0.245	0.222	0.210
3/5	0.267	0.240	0.179	0.200
3/11	0.170	0.240	0.170	0.280

by the volumetric method was higher and at other samplings it was lower than by the gravimetric method. These results seem to indicate that one great difficulty in determining the percentage of carbon dioxide in the soil air by gravimetric methods is the securing of a representative sample of soil air for analysis. When the concentration of carbon dioxide in the soil air is relatively low, the sample must be relatively large, and in taking a large sample it is difficult to get only the soil air.

In order to secure more information regarding the effect of size of samples of air and successive samplings on the concentration of carbon dioxide, soil tubes were planted in field soils early in June and after about 3 weeks samples were taken for analysis. In preliminary studies several procedures were compared, using potassium hydroxide and barium hydroxide, to absorb the carbon dioxide and determining it by gravimetric and titrametric methods. Finally, barium hydroxide in modified Truog towers was used to absorb the carbon dioxide. Two successive 4-liter samples were taken on June 26 by aspiration at the rate of 4 liters per hour. Two successive 4-liter samples were taken again on June 28, but the first 300 cc. were discarded. On July 1, 2 liters were discarded and then 2 successive 2-liter samples were taken for analysis. One 12-liter sample was taken on July 2. The results are given in table IV.

At the June 26 sampling, the percentage of carbon dioxide was 0.54 in the first sample and 0.32 in the second sample. At the next sampling, June 28, when 300 cc. were discarded before taking the samples for analysis, the percentage of carbon dioxide was approximately the same as at the first sampling, being 0.58 and 0.40 percent for the first and second 4-liter samples, respectively. The results checked at the third sampling, July 1, when the first 2 liters of air were discarded, but it is doubtful if they represent the concentration of carbon dioxide in the soil air. With a larger sample, 12 liters, on July 2, the percentage of carbon dioxide was 0.23 percent.

TABLE IV. CARBON DIOXIDE IN SUCCESSIVE SAMPLES OF AIR FROM FIELD SOILS.

Date	Sample no.	Volume of sample liters	Percent CO_2
6/26	1	4	0.54
	2	4	0.32
6/28*	1	4	0.58
	2	4	0.40
7/1†	1	2	0.32
	2	2	0.33
7/2	1	12	0.23

*First 300 cc. discarded.
†First 2 liters discarded.

In general these results show that the gravimetric and titrametric methods used are not suitable for determining the percentage of carbon dioxide in the soil air. It is difficult to secure a representative sample of the air for analysis. The rate of aspiration must be slow in order to secure complete absorption of the carbon dioxide and, when a large sample must necessarily be taken, too much time is required. If the gravimetric method is employed, difficulty is sometimes encountered with the drying agents. The method is quite unsatisfactory and cannot be recommended. Volumetric methods seem much preferable.

PART II. EVOLUTION OF CARBON DIOXIDE FROM SOILS

The evolution of carbon dioxide from the soil bears a relation to the rate of its production in the soil, but methods generally employed for determining the amount evolved from the soil have not shown this relation accurately. These methods have consisted in passing carbon dioxide-free air through or over the soil in cylinders, flasks or other suitable containers. While giving data which are of value in indicating the comparative effects of certain treatments, the conditions are rather arbitrary and the results cannot be considered to show the normal rate of carbon dioxide production in the soil. If free diffusion is the chief factor in the liberation of carbon dioxide from the soil (13, 14, 21), methods not involving the use of suction offer greater possibilities than the methods involving the aeration of the soil and the use of suction in taking the sample.

1. FREE EVOLUTION OF CARBON DIOXIDE FROM THE SOIL

It was considered desirable to study the influence of length of incubation period, size of sample and size of flask most suitable for the work. A manometric method was designed, adapting the Barcroft differential manometer for this purpose.

Preliminary tests on the rate of carbon dioxide evolution from soil contained in a closed flask indicated that it was exceedingly difficult to obtain representative samples of air from the flask for analysis by volumetric methods. Petersen (19) made the determination of carbon dioxide titrametrically in an effort to avoid this difficulty.

In the work reported here 100 grams of dry Tama silt loam which had been previously passed through a 2-mm. sieve were weighed into each of 2 flasks which had a capacity of 1,136 and 1,137 cc. and a third sample was weighed into a flask of 526 cc. capacity. The moisture content was adjusted to 20 percent. The flasks were fitted with a two-hole rubber stopper carrying

an inlet and outlet tube, the latter extending to the surface of the soil. The flasks were placed in the themostat at 25° C. and allowed to stand for 20 minutes when the amount of carbon dioxide in the flask air was determined. The inlet and outlet tubes were then closed and the soils were incubated for 16 hours, after which time samples of the air were taken for analysis. Samples were taken for analysis again after 285 hours.

The percentage of carbon dioxide in the flask air was determined with a Haldane volumetric gas analysis apparatus and the results secured were expressed as milligrams of carbon dioxide per 100 grams of dry soil per 24 hours. A sample calculation makes this clear.

Weight of moist soil	100 grams
Percent moisture	10
Amount of water added	10 cc.
Specific gravity of soil	2.5
Volume of soil	36 cc.
Volume of water	20 cc.
Volume of flask	1,136 cc.
Volume of air space in flask	1,080 cc.
Percent of CO_2 in flask air in the beginning	0.160
Percent of CO_2 in flask air after 16 hours	0.337
Volume of CO_2 after 16 hours	3.6396 cc.
Barometric pressure	736.4 mm.
Temperature	25° C.
Density of CO_2	1.6884
Mgm. CO_2 per 100 gm. dry soil per 24 hours	5.38

The results are given in table V.

The percentage of carbon dioxide in the individual samples in flask 1 varied from 0.26 percent to 0.41 percent, the average of 7 samples being 0.337 percent. In flask 2 the variations were not so wide and the average of 7 samples was 0.332 percent. While the individual samples varied considerably, the averages of a large number of determinations were sufficiently accurate for comparisons. The average rate of production was essentially the same in the large and small flasks during the first 16 hours. The average rate of production was found to be much smaller when the time period was 285 hours, the decrease being much greater in the small flask. This decrease in the rate of production may have been due to a decrease in the oxygen pressure in the flasks, to a detrimental effect of carbon dioxide which had accumulated in the flasks, to a decrease in the food supply, to a decrease in moisture or to a detrimental effect of an accumulation of metabolic products other than carbon dioxide. Although no determinations were made of the oxygen content of the flask air, it is rather unlikely that this decrease was due to a lack of oxygen, when the small amount used to produce the carbon dioxide found is considered.

Deherain and Demoussy (5) found that the presence of carbon dioxide in certain concentrations did not affect the rate of its production provided there was a sufficient supply of oxygen. Butjagin (4) concluded that the decrease in numbers of organisms in a closed system was not due to a deficiency of oxygen but very probably to the products of life activity. Kolkwitz (9) found that normal yeast fermentation was possible under relatively high pressure. It is also very unlikely that food and moisture were the limiting factors in production here. These questions are being studied further. Therefore, it would seem from these results that this decrease in the rate of production of carbon dioxide was due either to an accumulation of carbon dioxide or to the accumulation of metabolic products other than carbon dioxide.

The first sample of air taken from the flasks usually contained a lower percentage of carbon dioxide than succeeding samples because the carbon dioxide had not yet diffused into the tube which extended from the soil surface to the volumetric apparatus. In further experiments, the first 10 cc. of air were discarded but the successive samples did not check. The percentage of carbon dioxide in the samples also varied with the location from which the samples were taken. The air nearer the surface of the soil was richer in carbon dioxide than that taken midway between the top and the bottom of the flask.

TABLE V. THE RATE OF CARBON DIOXIDE EVOLUTION FROM SOILS.

Flask no.	Volume	Sample no.	Percent CO_2 After 16 hrs.	Mgm. CO_2 per 100 gm. soil per 24 hrs.	
				During 16 hrs.	During 285 hrs.
1	1,136cc.	1	0.26		
		2	0.41		
		3	0.34		
		4	0.34		
		5	0.34		
		6	0.34		
		7	0.33		
		Av.	0.337	5.38	2.25
2	1,137cc.	1	0.34		
		2	0.34		
		3	0.38		
		4	0.33		
		5	0.32		
		6	0.30		
		7	0.32		
		Av.	0.332	5.05	2.85
3	526cc.	1	0.54		
		2	0.62		
		3	0.62		
		4	0.62		
		5	0.61		
		6	0.54		
		7	0.56		
		Av.	0.588	5.22	0.98

This indicated that mixing the air in the flask would overcome the difficulty, and in the next experiments two methods were employed for mixing the air before sampling.

Two flasks were set up as in the preceding experiment and incubated 24 hours, after which the percentage of carbon dioxide was determined in the air in each flask, after discarding the first 10 cc. Three 10-cc. samples were taken successively and then the flasks were shaken vigorously for 1 minute, after which two more samples were taken successively. The results presented in table VI show that agreement betweeen the readings for successive samples was not secured by shaking the flasks.

TABLE VI. EFFECT OF SHAKING FLASK ON THE CARBON DIOXIDE IN SAMPLES OF AIR IN THE FLASK.

Flask no.		Sample no.	Percent CO_2
1	Before shaking	1	0.51
	Before shaking	2	0.74
	Before shaking	3	0.68
	After shaking	4	0.74
	After shaking	5	0.83
2	Before shaking	1	0.44
	Before shaking	2	0.51
	Before shaking	3	0.62
	After shaking	4	0.66
	After shaking	5	0.70

Another set of two flasks was set up using an untreated soil and a soil treated in the greenhouse with 300 pounds per acre of cyanamid. Four successive 10-cc. samples were taken from each soil after 48 and 72 hours and again after 7 days. Just before the 7-day samples were taken, the flasks were shaken vigorously for 1 minute. The results are presented in table VII. Apparently this shaking before sampling did not mix the air sufficiently and the duplicate samples did not check very closely.

TABLE VII. THE EFFECT OF SHAKING FLASK ON THE CARBON DIOXIDE IN SAMPLES OF AIR FROM THE FLASKS.

	Sample no.	Percent CO_2 after		
		48 hrs. unshaken	72 hrs. unshaken	7 days; flasks shaken vigorously
Check	1	0.17	0.20	0.68
	2	0.31	0.43	0.81
	3	0.35	0.46	0.47
	4	0.40	0.47	——
	Av.	0.31	0.39	0.65
Soil + cyanamid	1	0.30	0.62	0.64
	2	0.46	0.67	0.66
	3	0.42	0.54	0.72
	4	0.49	——	——
	Av.	0.41	0.61	0.67

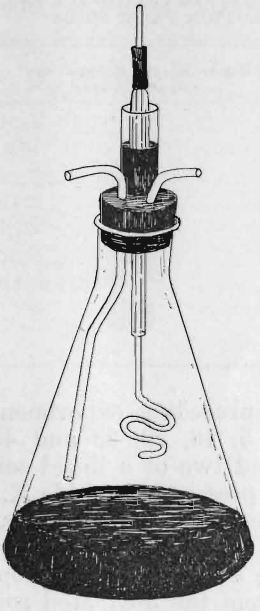

Fig. 2. Flask fitted with mercury seal stirrer.

Three series of two flasks each were set up, the one equipped with a stirrer (fig. 2) to determine whether or not stirring the air in the flask before taking the sample for analysis would mix the air sufficiently so that a representative sample might be obtained. The air was stirred for 5 minutes, using a stirrer attached to a small motor, before the samples were taken for analysis. The results presented in table VIII show that uniform readings were obtained when the air was stirred.

Then the average rate of carbon dioxide production at various intervals was made, in order to determine the length of the incubation period representing maximum rates of production.

Two 1-liter flasks fitted with stirrers and containing 100 grams of Tama silt loam at 23 percent moisture were placed in the thermostat at 25° C. After 20 minutes the carbon dioxide was determined and again after 6, 12, 24 and 30 hours. The results are presented in table IX.

TABLE VIII. THE EFFECT OF STIRRING THE AIR IN THE FLASK BEFORE TAKING THE SAMPLE FOR ANALYSIS.

Series no.	Sample no.	Percent CO_2		Mgm. CO_2 per 100 gm. soil per 24 hrs.	
		Air stirred	Air not stirred	Air stirred	Air not stirred
1	1	0.28	0.30		
	2	0.28	0.30		
	3	0.28	0.30		
	4	0.28	0.26		
	5	0.27	0.22		
	Av.	0.278	0.276	2.96	3.06
2	1	0.25	0.24		
	2	0.25	0.27		
	3	0.25	0.34		
	4	0.25	0.18		
	5	0.24	0.18		
	Av.	0.248	0.242	2.56	2.30
3	1	0.26	0.30		
	2	0.26	0.27		
	3	0.26	0.23		
	4	0.26	0.40		
	5	0.26	0.28		
	Av.	0.26	0.296	4.16	4.58

TABLE IX. THE EFFECT OF LENGTH OF INCUBATION PERIOD ON THE AVERAGE RATE OF CARBON DIOXIDE EVOLUTION FROM SOILS.

Sample taken	Flask no.	Mgm. CO_2 per 100 gm. dry soil per 24 hrs.
In the beginning	1	——
	2	——
After 6 hours	1	1.684
	2	1.686
After 12 hours	1	1.692
	2	1.684
After 24 hours	1	2.987
	2	2.972
After 30 hours	1	2.561
	2	2.718

Another test was made similar to the preceding experiment except that analyses were made after 5, 16, 22, 28 and 40 hours. Two flasks of an unlimed soil and two of a limed soil were set up. The results of this test are presented in table X.

The results secured in these two experiments indicate that the maximum rate of production was obtained after 16 to 24 hours in 1-liter Erlenmeyer flasks. The average results from the limed soil were lower than those from the untreated soil in all the tests.

It was noted in previous experiments that the rate of production of carbon dioxide was sometimes less (table V) with the same amount of soil in 500-cc. flasks than in 1-liter flasks. Three series of flasks were set up to determine the rate of production using the 500-cc. and 1,000-cc. flasks.

In series 1, 100 grams of dry Tama silt loam were weighed into each of two flasks, the one having a volume of 1,145 cc.

TABLE X. THE EFFECT OF LENGTH OF INCUBATION PERIOD ON THE AVERAGE RATE OF CARBON DIOXIDE EVOLUTION FROM SOILS.

Sample taken	Flask no.	Mgm. CO_2 per 100 gm. dry soil per 24 hrs.	
		Untreated soil	Soil treated with 3 T. lime per A.
After 5 hours	1	1.135	0.987
	2	——	0.962
After 16 hours	1	3.940	3.353
	2	2.842	3.095
After 22 hours	1	3.933	3.206
	2	2.963	3.256
After 28 hours	1	2.997	2.584
	2	2.328	3.047
After 40 hours	1	2.840	1.866
	2	1.912	2.139

and the other of 526 cc. The moisture content was adjusted to 24 percent and the soils were incubated at 25° C. for 24 hours, after which the percentage of carbon dioxide was determined and the average rate of production calculated. A second and a third series were made in a similar way. The results are given in table XI. In each series, the rate of production in the 500-cc. flask was about one-half as much as in the 1-liter flasks, except when 50 grams of soil were used in a 500-cc. flask, in which case the rate of production was higher than when 100 grams were used. Probably a shorter incubation period for the 500-cc. flasks would have given similar results to the 24-hour incubation period for the 1-liter flasks.

TABLE XI. THE EFFECT OF SIZE OF FLASK ON RATE OF CARBON DIOXIDE PRODUCTION.

Series no.	Volume of flask cc.	Mgm. CO_2 per 100 gm. dry soil per 24 hrs.
1	1,145	2.92
	526	1.69
2	1,136	3.76
	1,131	4.10
	526	1.33
3	1,136	3.61
	1,137	3.73
	526*	2.66

*50 gm. of soil.

2. EFFECT OF TREATMENT ON THE EVOLUTION OF CARBON DIOXIDE FROM SOILS BY THE FREE EVOLUTION METHOD

It was considered desirable to study the suitability of the above procedure for determining the effects of soil treatment on the rate of carbon dioxide production. The procedure finally adopted consisted of incubating 100-gram portions of soil in 1-liter Erlenmeyer flasks in the thermostat at 25° C., and a moisture content of 50 percent of saturation, stirring the air in the flask 1 minute and discarding the first 10 cc. before taking the sample for analysis. This procedure was followed in all cases, except the first where 100 grams of Waukesha silt loam were used in 500-cc. flasks.

a. EFFECT OF LIME AND STRAW ON CARBON DIOXIDE PRODUCTION IN WAUKESHA SILT LOAM

One hundred grams of Waukesha silt loam which was passed through a 2-mm. sieve were treated in 500-cc. flasks. Soil 1 was untreated, soil 2 received an application of 1 percent dry oat straw, the straw being finely ground and well mixed with the soil. Soil 3 received an application of 10 tons per acre of lime,

TABLE XII. THE EFFECT OF STRAW AND CALCIUM CARBONATE ON CARBON DIOXIDE PRODUCTION IN THE SOIL (WAUKESHA SILT LOAM).

Soil no. and treatment	Mgm. CO_2 per 100 gm. dry soil per 24 hrs.	
	During 24 hrs.	During 48 hrs.
1. Check	0.70	0.87
2. 1% dry oat straw	0.86	1.16
3. 3 T. lime per acre	0.93	0.88

chemically pure calcium carbonate being used. The moisture content of the soil was adjusted to 40 percent. The soils were incubated at 28° C. for 24 hours, after which time the percentage of carbon dioxide in the flasks was determined volumetrically and the rate of production calculated. The results are given in table XII.

The average rate of production of carbon dioxide was increased by treatment with straw and lime, the lime being more effective than straw during the first 24 hours; but there was no apparent stimulation by lime during 48 hours, whereas the straw had increased the rate materially.

b. EFFECT OF CALCIUM HYDROXIDE ON CARBON DIOXIDE PRODUCTION IN TAMA SILT LOAM

The effect of lime as calcium hydroxide on the rate of production of carbon dioxide in an acid Tama silt loam was determined in the next experiment. In one series of 1-liter flasks calcium hydroxide was applied to the soil at the rate of 3 tons per acre and the rate of carbon dioxide production determined. The treatment was made in duplicate and duplicate untreated soils were run as checks. In another series the same soil which had been treated 4 months previously was used and the rate of carbon dioxide production determined. The results are given in table XIII.

A decrease in the carbon dioxide content of the flask air occurred in one case and in another there was no increase when

TABLE XIII. THE EFFECT OF CALCIUM HYDROXIDE ON CARBON DIOXIDE PRODUCTION IN SOILS.

Treatment	Mgm. CO_2 per 100 gm. dry soil per 24 hrs.	
	Immediately after treatment	4 months after treatment
Check	1.08	1.93
Check	1.07	1.91
3 T. per A. $Ca(OH)_2$	0	5.95
3 T. per A. $Ca(OH)_2$	*	3.04

*There was less CO_2 in flask air after 24 hours than there was in the beginning.

calcium hydroxide was applied to the soil. After 4 months, however, there was a marked increase in the rate of carbon dioxide production in the limed soil.

c. EFFECT OF MANURE AND CROP RESIDUES ON CARBON DIOXIDE PRODUCTION IN CARRINGTON LOAM

The soils used in a study of nitrogen changes and microorganisms (25) were sampled 9 months after treatment and the rate of carbon dioxide production determined as in the above experiments. The results secured are given in table XIV.

The effect of these materials on the bacterial action in the soil is reflected in the rate of carbon dioxide production which is also correlated with the decomposition of the materials. The sweet clover roots, cornstalks, oat straw and adco-straw manure showed a much higher rate of carbon dioxide production, indicating that the other materials were slower to decompose.

TABLE XIV. THE EFFECT OF VARIOUS MANURES ON CARBON DIOXIDE PRODUCTION IN THE SOIL.

Pot no.	Treatment	Mgm. CO_2 per 100 gm. dry soil per 24 hrs.
1	Check	0.42
2	Farm manure	1.47
3	Adco-Straw manure	3.74
4	Adco-Stalk manure	0.65
5	Ammonium sulfate - straw manure	1.58
6	Oat straw	2.33
7	Cornstalks	3.50
8	Sweet clover tops	1.11
9	Sweet clover roots	5.07

d. EFFECT OF STERILIZATION OF SOIL ON CARBON DIOXIDE PRODUCTION IN CARRINGTON LOAM

The rate of carbon dioxide production in sterile and non-sterile soil was studied in two series of soils. In series 1, duplicate 100-gram samples of Carrington loam were weighed into 1-liter Erlenmeyer flasks and sterilized in the autoclave 3 hours at 15 pounds steam pressure, cooled to 25° C. and incubated for 72 hours. Duplicate, non-sterile samples were incubated and served as checks. The percentage of carbon dioxide after incubation was determined and the rate of carbon dioxide production calculated. The results showed that more than 90 percent of the carbon dioxide produced was due to the microorganisms. The rate of production in the non-sterile checks was 1.94 mgm. per 100 grams of dry soil per 24 hours, whereas it was only 0.15 mgm. in sterile soil. Very similar results were secured with the Tama silt loam. The results showed also that large amounts of carbon dioxide were formed in sterilization.

3. EFFECT OF CROP ROTATION ON CARBON DIOXIDE PRODUCTION IN SOILS

The continuous corn plots at the Agronomy Farm were sampled on April 27, May 13 and May 30 and the rate of carbon dioxide production determined as in the preceding experiments. Preliminary work was done on the technique of sampling field soils. In sampling laboratory and greenhouse soils in the preceding experiments satisfactory results were secured by using a composite sample for analysis. It was thought desirable, however, to study the rate of production from samples taken from several different locations on the same plot compared with composite samples. A borer similar to the one used by Lundegardh (14) was used to secure a definite volume of soil. It was found that the results varied considerably for samples taken at short distances, whereas duplicate composite samples usually were in close agreement. In the studies on field soils, therefore, composite samples were employed.

At the first sampling the ground had been harrowed just previous to planting. At the second sampling the corn was just up and at the last sampling the corn was about 1 foot high. Plots 905 and 906 are located on Carrington loam while plots 908 and 909 are on Carrington sandy loam. The results secured on the two soil types are not entirely comparable but the effect of manure on the rate of carbon dioxide production in Carrington loam is shown in the results and of lime on Carrington sandy loam. The data are shown in table XV.

TABLE XV. THE EFFECT OF SOIL TREATMENT AND CROPS ON THE RATE OF CARBON DIOXIDE PRODUCTION IN THE SOIL—CONTINUOUS CORN.

Plot no.	Treatment	Mgm. CO_2 per 100 gm. dry soil per 24 hrs.		
		4/27	5/13	5/30
905	Check	1.45	3.41	1.36
906	Manure	4.07	4.18	2.12
907	Manure + lime	1.90	——	1.62
908	Lime	2.63	1.85	1.32
909	Check	1.50	1.63	1.29

Composite samples were taken on May 26 and June 2 from the check plot, the manured plot and the plots receiving manure and lime in the 4-year rotation. These plots are located on uniform Clarion loam. The results secured are given in table XVI.

The rate of production of carbon dioxide was higher at the first sampling but decreased more rapidly in the check soils

than in the manured soil. Manure was more effective than manure and lime in increasing the rate of carbon dioxide production in this soil at this time.

TABLE XVI. THE EFFECT OF SOIL TREATMENT AND CROP ON THE RATE OF CARBON DIOXIDE PRODUCTION IN THE SOIL—4 YEAR ROTATION.

Treatment	Mgm. CO_2 per 100 gm. dry soil per 24 hrs.	
	5/26	6/2
Check	3.83	2.62
Manure	4.49	4.06
Manure + lime	4.26	3.12

PART III. MANOMETRIC METHOD FOR DETERMINING THE RATE OF CARBON DIOXIDE PRODUCTION IN SOILS BY MEASURING OXYGEN CONSUMPTION

Iwanoff (7) studied carbon dioxide production in alcoholic fermentation by means of a fermentation manometer. The carbon dioxide formed was measured by the pressure it exerted when kept at a constant volume. An attempt was made to adapt this method to the study of carbon dioxide production in soils. No pressure differences, however, were obtained even when the manometer was placed on a slant with a slope of 1 centimeter in 1 meter.

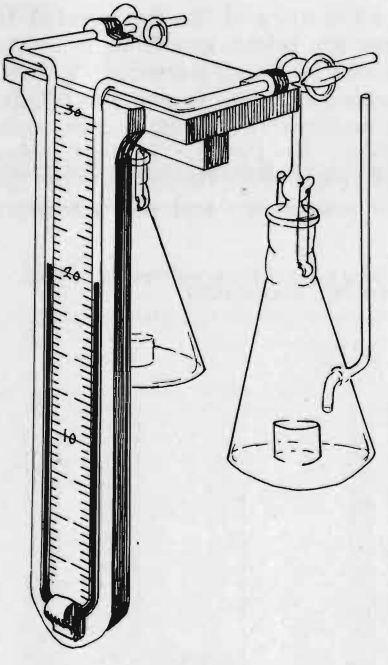

Fig. 3. Modified Barcroft differential manometer.

A Barcroft manometer (fig. 3) was adapted for this purpose by substituting 500 cc. flasks with a small chamber sealed in the bottom for the soil and a side tube for taking a sample of the air for analysis instead of the small cups ordinarily used in bacteriological work.

Ten grams of dry soil were put in the small chamber of the right cup and 10 cc. of a 35 percent potassium hydroxide solution in the flask surrounding the soil. Three cc.

TABLE XVII. THE RATE OF CARBON DIOXIDE PRODUCTION IN THE SOIL—CUPS NOT CO_2-FREE IN THE BEGINNING.

Time of reading after beginning; hours and minutes (X)	Mgm. CO_2 per 10 gm. dry soil (Y)	Y/X
1:00	0.295	0.2950
1:15	0.324	0.2592
1:30	0.518	0.3453
1:45	0.712	0.4068
3:00	1.392	0.4640
4:00	1.553	0.3882
5:00	1.785	0.3570
6:00	1.785	0.2975
7:00	1.942	0.2744
22:40	3.106	0.1370
23:10	3.365	0.1453
23:40	3.365	0.1422
24:10	3.430	0.1420
25:00	3.527	0.1410
25:30	3.560	0.1396
26:00	3.592	0.1381
27:00	3.656	0.1354
28:00	3.721	0.1328
29:00	3.786	0.1305
30:00	3.851	0.1283
31:00	3.948	0.1273
345.66	53.213	SY/SX = 0.1539

of distilled water were added to the soil and the cup aerated 10 minutes with carbon dioxide-free air before attaching it to the manometer. The left cup contained 10 cc. of potassium hydroxide and was aerated before attaching it to the manometer. Soda-lime tubes were attached to the manometer as guard tubes while the cups were open. The manometer was then set in the thermostat for 1 hour at 25° C., then the cocks were turned to place the cups in connection through the manometer and the barometer

TABLE XVIII. THE RATE OF CARBON DIOXIDE PRODUCTION IN THE SOIL—CUPS CO_2-FREE IN THE BEGINNING.

Time of reading after beginning; hours and minutes (X)	Mgm. CO_2 per 10 gm. dry soil (Y)	Y/X
1:07	0.097	0.0870
2:00	0.194	0.0970
2:52	0.258	0.0902
3:37	0.380	0.1051
4:37	0.483	0.1046
7:17	0.710	0.0975
7:37	0.807	0.1060
9:00	0.904	0.1004
19:37	2.110	0.1075
20:00	2.388	0.1194
21:00	2.388	0.1137
22:00	2.549	0.1158
23:00	2.710	0.1176
24:00	3.033	0.1263
28:00	3.388	0.1210
196.73	22.399	SY/SX = 0.1144

TABLE XIX. THE RATE OF CARBON DIOXIDE PRODUCTION IN THE SOIL.

Time of reading after beginning; hours and minutes (X)	Mgm. CO_2 per 10 gm. dry soil (Y)	Y/X
1:15	0.065	0.0520
2:00	0.130	0.0650
3:30	0.260	0.0742
4:22	0.325	0.0744
5:22	0.325	0.0605
6:00	0.390	0.0650
7:00	0.520	0.0743
7:45	0.520	0.0671
22:15	1.575	0.0708
23:00	1.656	0.0724
24:00	1.690	0.0704
25:00	1.721	0.0688
26:00	1.819	0.0699
27:15	1.884	0.0691
28:00	1.916	0.0685
29:30	2.014	0.0719
30:15	2.047	0.0676
30:45	2.144	0.0697
46:30	2.794	0.0600
47:30	2.859	0.0601
49:00	2.924	0.0597
446.2	29.578	SY/SX = 0.0662

reading taken. The manometer readings were made at intervals for several hours and the amount of carbon dioxide produced calculated. The results secured are presented in tables XVII, XVIII, XIX and XX and are typical of several sets of results secured with this soil.

The results presented in table XVII were secured without first freeing the flask of carbon dioxide while the results presented in tables XVIII, XIX and XX were secured by first freeing the air in the flask of carbon dioxide. Samples of air taken from the side tube at different intervals showed complete absorption of the carbon dioxide produced, when 35 percent potassium hydroxide was the absorbent. With barium hydroxide as the absorbent, however, the carbon dioxide was never completely absorbed.

TABLE XX. THE RATE OF CARBON DIOXIDE PRODUCTION IN THE SOIL UNDER CONSTANT ENVIRONMENTAL CONDITIONS.

After	Mgm. CO_2		Mgm. CO_2 per hour	
	Manometer		Manometer	
	I	II	I	II
24 hours	2.03	2.05	0.095	0.084
48 hours	7.46	6.61	0.155	0.138
72 hours	13.10	12.82	0.182	0.178

DISCUSSION AND SUMMARY

The percentage of carbon dioxide in the soil air was determined in field soils and in the laboratory by gravimetric, titrametric and volumetric methods. The gravimetric and titrametric methods for determining the percentage of carbon dioxide in the soil air were found to be entirely unsatisfactory for a number of reasons, chief of which was the difficulty in securing a representative sample of the air for analysis. The volumetric method was found to give fairly satisfactory results, especially in the laboratory. The large Haldane gas analysis apparatus fitted with a special burette calibrated to 0.01 cc. proved more satisfactory as a portable apparatus than the volumetric apparatus designed by Lundegardh. A soil tube made of brass and having an air space of about 300 cc. which was placed in the soil at the desired depth and left there was more satisfactory than the pointed brass tubes which are pushed into the soil just before taking the sample.

The free evolution of carbon dioxide from the soil was found to be a suitable method for studying the effects of certain soil treatments on the rate of carbon dioxide production in soils. A further study of the method is being made comparing it with other methods formerly employed for that purpose. In the free evolution method the 1-liter Erlenmeyer flask and 100-gram sample of soil are convenient, but larger or smaller samples may be used and smaller flasks may be employed also provided the incubation period is proportionately shorter. Uniform samples for volumetric analysis can only be obtained by stirring the air in the flask before sampling.

The results secured by the gravimetric method for determining the amount of carbon dioxide did not check with the volumetric analysis. This was no doubt due to the amount and rate of aspiration. The gravimetric methods in general are not so well adapted to this work and the volumetric method is recommended where it is possible to use it.

The Iwanoff fermentation manometer was found unsuitable for measuring the rate of carbon dioxide production in soils. The results secured indicate that in soils an equivalent volume of oxygen is absorbed for a given amount of carbon dioxide produced and, therefore, an increase in pressure in the Iwanoff manometer with soils under aerobic conditions would not be expected.

The Barcroft differential manometer appears to be suitable for measuring the rate of carbon dioxide production in soils and possesses the advantage that one can follow the rate from time to time. The results secured by this method are consistently higher than those obtained by the free evolution

method. This is probably due to the smaller samples of soil and better aeration.

Lemmermann and Wiessmann (12) studied carbon dioxide production in soils variously treated. The soils were put in flasks and carbon dioxide-free air passed through the flask, the carbon dioxide evolved being absorbed in soda-lime and weighed. They concluded that carbon dioxide production in soils proceeds according to the equation $X=akt^m$ in which X equals the quantity of carbon dioxide produced in time t, a is the initial carbon dioxide content of the soil and k and m are constants. The results secured with the differential manometer, however, bear a striking resemblance to the curve of the life phases in a bacterial culture and indicate that carbon dioxide production in the soil under constant environmental conditions proceeds in accordance with the growth law of bacteria.

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DAIRY INDUSTRY. M. Mortenson, B.S.A., Res. Prof. and Head; B. W. Hammer, B.S.A., Ph.D., Res. Prof.; E. W. Bird, B.S., Ph.D., Res. Asst. Prof.; R. V. Hussong, B.S., Res. Asst.; D. F. Breazeale, B.S., M.S., Res. Asst.; Ralph Farmer, B.S., M.S., Res. Asst.; C. H. Meyer, B.S., M.S., Res. Asst.

ENTOMOLOGY. Carl J. Drake, B.S., B.Ped., M.A., Ph.D., Res. Prof. and Head; C. H. Richardson, A.B., M.S., Ph.D., Res. Prof.; O. W. Park, B.S., M.S., Ph.D., Res. Assoc. Prof. G. C. Decker, B.S., M.S., Ph.D., Res. Asst.; Bernard Travis, B.S., Res. Asst.; H. D. Tate, B.S., M.S., Res. Asst.; Roy A. Grout, B.S., M.S., Res. Grad. Asst.; G. A. Thurber, B.S., Res. Grad. Asst.

FARM CROPS AND SOILS. P. E. Brown, B.S., A.M., Ph.D., Res. Prof. and Acting Head.

FARM CROPS: H. D. Hughes, B.S., M.S.A., Res. Prof. and Head; L. C. Burnett, B.S.A., M.S., Res. Prof.; J. B. Wentz, B.S., M.S., Ph.D., Res. Assoc. Prof.; J. L. Robinson, B.S., M.S., Supt. of Exp'ts. F. S. Wilkins, B.S., M.S., Res. Asst. Prof.; L. D. Eagles, B.S., Field Foreman; M. T. Jenkins, B.S., M.S., Ph.D.; A. A. Bryan, B.S., M.S., Ph.D., and R. W. Jugenheimer, collaborators.

SOILS: P. E. Brown, B.S., A.M., Ph.D., Res. Prof. and Acting Head; F. B. Smith, B.S., M.S., Ph.D., Res. Assoc. Prof.; R. H. Walker, B.S., M.S., Ph.D., Res. Assoc. Prof.; T. H. Benton, B.S., M.S., Res. Asst. Prof.; C. L. Orrben, Res. Asst. Prof.; H. R. Meldrum, B.S., Res. Asst. Prof.; A. J. Englehorn, B.S., M.S., Res. Asst. Prof.; R. E. Bennett, B.S., Res. Asst. Prof.; L. W. Forman, B.S., M.S., Supt. of Exp'ts.

GENETICS. E. W. Lindstron, A.B., Ph.D., Res. Prof. and Head; W. V. Lambert, B.S., M.S., Ph.D., Res. Asst. Prof.

HOME ECONOMICS. Genevieve Fisher, B.S., A.M., Head.

FOODS AND NUTRITION: P. Mabel Nelson, M.S., Ph.D., Res. Prof. and Head; Pearl Swanson, M.S., Ph.D., Res. Prof.; Bessie Hammer, B.S., Res. Asst.; Gladys Timson, B.S., Res. Asst.; Mabel Gunson, Technician.

HOUSEHOLD EQUIPMENT: Louise J. Peet, M.S., Ph.D., Res. Prof. and Head; Lenore Sater, B.S., M.S., Res. Asst. Prof.

TEXTILES AND CLOTHING: Rosalie Rathbone, B.S., M.A., Head; Rachel H. Edgar, M.S., Ph.D., Res. Prof.

HORTICULTURE AND FORESTRY. B. S. Pickett, B.S.A., M.S., Head.

POMOLOGY: T. J. Maney, B.S., Res. Prof. and Head; H. L. Lantz, B.S., M.S., Res. Asst. Prof.; H. H. Plagge, B.S., M.S., Res. Asst. Prof.; V. T. Stoutemyer, B.S., Res. Asst.

VEGETABLE CROPS: A. T. Erwin, B.S., M.S., Res. Prof. and Head; E. S. Haber, B.S., M.S., Res. Asst. Prof.; N. D. Morgan, B.S., Res. Asst.

FORESTRY: G. B. MacDonald, B.S.F., M.F., Head.

FLORICULTURE: E. C. Volz, B.S., M.S., Head.